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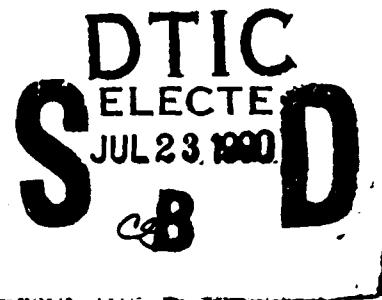
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## THE EFFECT OF HEATING ON TENDON AND JOINT BLOOD FLOW

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## NOTICES

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.



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19. ABSTRACT (Continued)

flow. It appears that even if there was local shutdown of perfusion in a small area adjacent to the one being observed, the perfusion in adjacent areas (within 1 mm) is sufficient to prevent supersaturation with inert gas and hence the formation of bubbles in these tissues.

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## THE EFFECT OF HEATING ON TENDON AND JOINT BLOOD FLOW

### BACKGROUND

It has been recognized for several years by both U.S. and Soviet research scientists (1) that pilots and astronauts must prebreathe oxygen for at least 4 h to prevent decompression sickness during extravehicular activity (EVA). Further, in most instances, the decompression sickness is of the limb bends type (aviator's bends), and does not affect the central nervous system.

Aviator's bends differs from divers decompression sickness in which there may be not only a large percentage of limb bends, but also a significant amount of central nervous system decompression sickness such as spinal cord weakness or paralysis or numbness of the limbs.

In both types of activity, the individual has been exposed to reduced atmospheric pressure. However, in the case of the diver, he works under elevated atmospheric pressure during which time the body is not supersaturated with inert gas. Supersaturation of the body with inert gas occurs only during and after decompression. In contrast, the astronaut or pilot may engage in heavy exercise after being decompressed to high altitude or EVA environment. While he is working, the body is supersaturated with inert gas. It is well recognized that exercise after decompression, whether from underwater to sea level, or from sea level to altitude, will exacerbate the creation of bubbles, both in the tissues and in the circulation.

Thus, when the diver returns to the surface he creates the prerequisites for bubble formation, but since he does not exercise at that time he will minimize the danger of their formation. On the other hand, if the pilot or astronaut has not eliminated most of the nitrogen from the body by prebreathing oxygen, some tissues may still be supersaturated with inert gas at space suit pressures. This condition is worsened by the often vigorous activity required at altitude and which tends to create bubbles during EVA. Fortunately, in many instances a pilot who suffers decompression sickness at altitude becomes symptom free upon return to ground. It is, however, a serious matter if decompression sickness occurs in a space station since treatment requires a large amount of oxygen, best delivered under pressure.

It is presumed that limb bends is caused by bubbles in tissues (although circulating bubbles also may be present) (1). This fact raises a fundamental question; namely, how can enough nitrogen remain in the body to cause this problem after 4 h of oxygen breathing.

Because the location of the pain is usually in the joints and tendons, it is logical to assume that the problem may be due to a significant amount of residual nitrogen remaining in these tissues even after extensive prebreathing of oxygen. Since it is usually assumed that bubbles are the cause of the pain, it also must be assumed that these bubbles are located in the vicinity of nerve endings. However, even if the bubbles are near nerve endings they would not necessarily cause pain unless they press on these nerve endings, especially if the tissue is "tight" tissue which is defined as dense with little elasticity.

Tendons and joint capsules meet the requirements of "tight" tissues. They contain fibrous connective tissue and collagen and are very compact in structure. Their fibers resemble rope in certain locations. The fibers are twisted about themselves so that when they pass over joints they will remain round and compact, distributing the stresses evenly throughout the entire tendon cross section (2). It follows, therefore, that there is virtually no room for bubbles to develop in this type of structure without causing some displacement of the tissues which distort and stimulate the nerve endings located there.

While this theory can account for the location of pain in the event of bubble formation in tight tissues, the following question must be raised: why should bubbles develop in these areas after such extended oxygen prebreathing periods? In fact, this type of bends is so typical in pilots that it is sometimes referred to as "aviator's bends".

Hills (3) has suggested an explanation for this localization of pain in "tight" tissues. He pointed out that tendons and joint capsules should have a much lower metabolic rate than muscle or most other body tissues because their primary action is to sustain passive stress, and not to engage in active energy-consuming contraction. If this is so, then it might be expected that these tissues also would require a lower perfusion rate than other body tissues, and the capillaries could remain closed for longer periods.

To study this hypothesis, Hills examined the Achilles tendons of frogs and guinea pigs. After exposing the tendons, he injected filtered carbon particles into the inferior vena cava. Thus, only the smallest carbon particles passed through the lungs and circulated freely in the systemic circulation. He observed that at varying periods after injection, (often after 30-60 min), discrete sections of the tendons suddenly became dark, showing that the capillary bed had just opened, and allowed the carbon granules to enter and lodge there. He reported observing that the longest delay before perfusion opened was 4 h in one instance.

He also noted that not all of the tendon became dark at the same time. Some areas several  $\text{mm}^2$  remained white for varying periods while adjacent areas became dark. There was very little overlap between adjacent areas causing a mottled appearance.

Thus, Hills demonstrated that, while the action of capillary opening and closing in tendons follows the opening and closing pattern originally described by Krogh (4) for skeletal muscle capillaries, the frequency of opening and closing cycles in tendons appeared to be much slower than in muscle. For example, he found that in guinea pigs, the periods of no flow averaged 43 min while Burton and Johnson (5) found that in muscle, the blood flow velocities varied by a factor of 10:1 within a 1-min interval, and in some instances, by a 100% variation within 5 s. Further, Johnson and Wayland (6) observed complete shut-down of blood flow for only a 30 s period in cat mesentery and skeletal muscle.

It should be noted, however, that in the studies by Burton and Johnson (5), as well as by Johnson and Wayland (6), theirs were in-vitro tissue preparations. The removal of such tissue was a serious insult which altered the blood supply and placed the tissues in a most artificial

environment. This does not mean that these observations were invalid, but that they must be viewed with the knowledge that the function of these tissues was lacking some physiological support.

Hills' in-vivo model may not suffer quite as severely from manipulation, although a question must be raised as to the effect of even minor manipulation, as well as the general effect of injecting foreign particles into the circulation. In our hands, the injection of even filtered carbon particles in the venous system of pigs resulted in some pulmonary hypertension, pulmonary distress, and a drop in perfusion. Further, the injection of these particles was accompanied by the injection of additional fluid which would change the blood flow characteristics. In an animal with a small blood volume, this may be a significant insult in itself.

For many years, based in part on the work of Burton, it has been taught that while only about 0.5% of the capillaries of the body are open at the same moment, they all tend to open periodically on a cycle of only a few seconds or minutes (7). The subject of low flow states in the microvessels in cats was discussed by Eriksson and Lisander (8) who noted that blood flow is rarely interrupted for more than a few minutes at most. Thus, Hills' observations of long closing times came as a surprise.

The importance of Hills' observation, if true, is interesting in view of the fact that both U.S. and Soviet astronauts found it necessary to prebreathe oxygen for at least 4 h to insure that they would not suffer decompression sickness when working in the EVA environment (1). If there are tissues in the body which have such a slow half-time, and which can sequester inert gas in sufficient quantity, then obviously, there would have to be changes made in the algorithms used in developing staged decompression tables for diving, as well as in the prebreathing time required prior to EVA for pilots and astronauts.

The problem of diving decompression tables can easily be solved by adjusting the time at various stops during ascent with only an economic penalty to pay. However, since it is likely that astronauts and space pilots may not always have the luxury of extending prebreathing time due to some operational mandate, it is imperative to confirm first of all that the joint and tendon blood flow theory is valid. If it is determined that the tendons and joint capsules are at fault in altitude bends, it is urgent to search for a way to increase the speed of denitrogenation in preparation for EVA operations or high altitude flying.

#### RATIONALE

The observations by Hills were, to us, unexpected, and they did not seem logical in view of the rather satisfactory tendon vasculature described by Field (2). It is true that when the tendon is cut, no profuse bleeding usually occurs although small individual vessels in the tendon appear to bleed freely whenever cut. On the other hand, it must be acknowledged that Hills is a very careful worker and his observations were quite convincing. Further, the 4-h close-down of the capillary bed which Hills reported in one case was in consonance with the findings of both NASA and the Soviets on the need for a 4-h prebreathing period (1).

If these observations were indeed valid, it seemed clear that there would be no way to overcome the requirement for a 4-h prebreathing period unless a certain incidence of decompression sickness could be accepted, or unless a way could be found to increase the length of the "on" portion of the perfusion cycle in these tissues.

Therefore, we proposed, first of all, to try to confirm Hills' observations using an entirely different technique. Second, whether the blood flow is shut down or simply reduced, we proposed to study the possibility of increasing the temperature of tendons and joint capsules and in this way increase the cellular metabolic requirements and, therefore, the rate of perfusion in those areas.

Initially, an effort was made to employ the hydrogen washout method using the Fick principle (9) as applied by Austrand (10) using molecular hydrogen as the tracer. This procedure was applied by implanting a hydrogen electrode in the tendon or capsule. Then the animal breathed a mixture of hydrogen and oxygen until there was a significant amount of hydrogen dissolved in the tissue under observation. By removing the hydrogen from the inspired gas, the hydrogen in the tissue would be removed by the circulation. As demonstrated by Yimenez (11) and others, it is possible by the Fick principle to calculate that blood flow in cc/gram of tissue per minute. While this is not a direct measurement, it has been shown to closely approximate the values measured more directly.

Unfortunately, tendon perfusion was so low that it was not possible to quantify blood flow in that tissue in a repeatable manner. The reason for this inconsistency apparently was that the amount of hydrogen was so small due to the low flow and to the low solubility of hydrogen in the tissue that the current created by the hydrogen was buried in the electronic noise of the system. Further, the hydrogen electrode proved to be sensitive to temperature changes, thus making it impossible to study the effect of heat on joint or tendon perfusion.

It was then decided to use a method which would not require major violation of the tissue being measured, and at the same time provide a way of continuously measuring very low flows. Thus, the laser-Doppler flow meter was selected.

This system employs a 2 mW Helium-Neon laser which produces a monochromatic light beam at a wavelength of 632.8 nm. The light is transmitted by an optical fiber to a probe which is placed near the tissue being observed. Some of the light is reflected back from moving red blood cells and undergoes a frequency shift (Doppler effect), while some of it is reflected from non-moving tissue which remains at the original frequency. The backscattered light, both Doppler-shifted and unshifted, is led by two optical fibers to two photodetectors (p-i-n diode photosensitive elements (12)), where the signal is processed and amplified to achieve a high signal-to-noise ratio. The reflected, unchanged light and the laser-Doppler-shifted light then are beat against each other, resulting in a third signal which reflects the difference.

The output signal is linearly related to the number of red cells multiplied by their velocity in the hemispheric measured volume (13). In most tissues, the measured surface is about 1.2-2 mm<sup>2</sup>(12), while the light

penetrates approximately 1.5 mm deep into the tissue, although Overaasen (14) observed that, in the urinary bladder of the calf, there was still 37% of the light present at a depth of 2.3 mm. The depth of penetration is somewhat dependent upon the color and density of the tissue being observed (15). Based on studies in skin, it appears that the 1 mm figure more closely reflects the situation in tendon and joint capsule.

The maximum sensitivity of the probe is obtained when it is about 0.6 mm from the tissue surface (15). If the probe is touching the tissue, there is a tendency for an overlap of the transmitting and receiving fiber apertures. This overlap causes insufficient light to be reflected, whereupon a message to this effect appears on the screen. At a probe distance of approximately 2 mm, the sensitivity decreases exponentially and again a message appears announcing a low-light level. In actual practice, there is sufficient latitude in the permissible distance between the probe and the tissue to achieve stable operations.

A number of studies have been made on different tissues including skin (16), femoral head (17), intestinal mucosa and muscularis (18), the heated foot (19), skeletal muscle (20), as well as many others. Tests have shown that there is a linear response to flows of low and moderate red cell velocities (21) when flow rates are within the physiological range for microvascular beds for tendons, skin, etc.

A question which must be raised concerns the safety of the laser beam used in this device. The maximum permissible laser exposure to skin is 2000 W/m<sup>2</sup>, distributed over 8 h, in any 24 h time period. This method does not exceed 570 W/m<sup>2</sup>. Since the power emitted from this unit's probe is 1 mW, and is distributed over a diameter of about 1.5 mm, it gives a power intensity of about 570 W/m<sup>2</sup>. Thus, it is well below Maximum Permissible Exposure (MPE), and may be operated well within the limits of safety to both humans and animals.

#### METHODS

This study involved 20 pigs, 5 dogs, and 4 goats. The protocols were approved by the Texas A&M Laboratory Animal Use Committee. The number of pigs used in this study was much larger than called for in the plan because the laser-Doppler technique has only recently been developed and it was necessary to determine its limitations and capabilities. It was especially important to eliminate the possibility that gross movement, respiration, or other artifacts might be confused with perfusion. Pigs received an IM pre-induction of Ketamine 20 mg/kg. As soon as the animals were heavily tranquilized, a vein was cannulated with a 27 gauge needle and Nembutal (60 mg/ml) with heparin was introduced. Each animal was titrated by this route to obtain a proper level of anesthesia, a level which was maintained throughout the experiment by additional Nembutal. The plane was maintained so that there was no shivering or other spontaneous body movement except quiet respiration. During the study the animals were also given, as needed, an IV infusion of a cocktail containing 2 mg Rompun with 0.1 mg Acepromazine per pound of body weight. This combination of tranquilizers appears to be a reliable method for maintaining the animal in a good plane of anesthesia with a minimum amount of Nembutal. It is recommended by veterinary anesthesiologists as causing the least amount of toxicity and alteration of the cardiovascular system. Our general experience is that these drug doses cause only minor pressure changes,

and that the reflexogenic areas of the cardiovascular system appear to remain functional. Furthermore, measurement of skin perfusion in the anesthetized pig indicates that, even under the above described anesthesia, perfusion remains nearly the same as in the conscious animal.

In passing, it may be well to note for future reference that in studies carried out on pigs for another later project, it was found that the use of Rompun and Acepromazine was not necessary. Excellent results may be obtained by an initial induction with Ketamine to implant the venous catheter, followed by an occasional infusion of Ketamine. If this new procedure is followed it will reduce the Nembutal needed to about one-third that previously required.

In the case of goats and dogs, the anesthesia protocol was similar to that used on the pigs except that the initial induction consisted of an IM injection of 30 mg Rompun, followed by the Nembutal.

**Tendon surgical preparation:** Animals were placed on either side and the Achilles tendon on the dependent leg was exposed. The tendon sheath was opened and removed in the area to be observed. Care was taken to select an area where no arteries or veins could be seen. A special effort was made to assure that the tendon was not under tension by allowing the leg to be relaxed. Since the animals were supine, the tendon was located at approximately heart level to avoid the effects of gravity on either arterial pressure or venous return. To prevent the possible effects of temperature fluctuations on the body, the animal was maintained at a room temperature of 72° F.

When the area had been prepared, two loose ligatures were passed under the tendon, one above and one below the location to be observed. These loose ligatures were lightly sewn to the probe holder to assure that the probe would remain directly over the tendon, but without strain. In addition, the probe holder was loosely sewn to the skin so that its center would remain precisely over the area of the tendon to be studied, and at the same time avoid placing tension on the two ligatures which also aided in positioning the tendon and probe. In some instances, a 4 X 4 cotton sponge was passed under the tendon to help hold it in the proper place, but again avoiding placing tension on the tendon.

The probe was placed between 0.5 and 1 mm above the tissue, although it has been shown by others (23) that the space between the probe and the tendon could be up to 3 mm without significantly altering the readings.

An effort was made to cover the exposed tendon outside the plastic probe holder so that there would be less chance of the tendon drying, experiencing a change of temperature, or being exposed to sudden drafts.

The laser-Doppler probe was initially calibrated by placing it in a holder which excluded outside light, thus allowing the laser light to reflect from a dull white surface which had no movement. This became the baseline which represented no blood flow. In most instances this value was from 0 to 0.2 perfusion units (PU). Initially, this value was checked by clamping the tendon above and below the position of the laser probe. The value to which the laser-Doppler meter dropped was considered to be the actual zero flow. It was considered that this cancelled the effect of Brownian movement (if such

existed), and the natural noise of the system. This method was used by Damber et al. (23). It was usually within 1 to 2 PU of the baseline and did not essentially alter our conclusions.

After the baseline was established, the probe was inserted in the holder which was over the tendon. It was allowed to remain without any manipulation for from 30 min to as long as 5 h. In some studies, the probe was occasionally moved to another holder located on the shaved flank of the same animal to measure skin blood flow. Care was taken not to abrade the skin during shaving to prevent triple response or other abnormal alterations of skin blood flow.

At the proper time, heat was applied in one of two ways. The first was via a heating coil which was built into the probe holder. This heater could be adjusted to temperatures from 30-45°C, in 2-deg increments. The second way was by using a heating pad which could cover either the local area of the probe, or the entire leg of the animal. Each heating study was continued without interruption for as long as 2 h with constant recording of blood flow.

To provide a visual display of perfusion, the infusion of filtered carbon particles was carried out following the technique described by Hills (3). In addition, fluorescein dye was used since this does not cause emboli, and can be observed with ultraviolet light. This dye is commonly used on humans for the study of skin graft viability since there is no known adverse physiological effect.

At the termination of some studies, the tendon was clamped above and below the probe to manually interrupt all blood flow to the area. The PU units dropped to approximately zero. The section of the tendon was surgically removed with the probe intact and in place. In this way it was possible to confirm that the probe had recorded actual perfusion and that the measurements were not artifacts. It also aided in establishing a true zero blood flow to check against the zeroing block which also was used.

Since the Doppler principle actually measures velocity of a surface, which will shift the frequency of the reflected laser beam, the question must be raised whether some of the Doppler shift is due not to red cell movement, but to the movement of the tendon itself.

The Doppler shift was studied in the following manner: The animal first was tranquilized heavily, followed by an infusion of Nembutal titrated to cause respiratory arrest. At this time the animal was in a complete state of muscle relaxation. It was then placed on a respirator which delivered air at the minimum pressure needed to cause gas exchange in the lungs.

This procedure did not eliminate changes in the laser-Doppler perfusion readings which were synchronous with the action of the respirator. However, it was noticed that when the animal was breathing with its own respiratory muscles, the rise in PU values came during expiration. If the positive pressure respirator was used, the rise in PU values was synchronized with inspiration. When the respirator was stopped and ventilation ceased, the PU values immediately dropped to a low level but still did not reach zero. The significance of these PU values will be discussed in the Results section.

It was difficult to dissect out the Achilles tendon in the pig. It is rather short and muscle fibers are inserted for some distance into the tendon. There was only a short length of tendon which appeared to be free from imbedded muscle fibers. Further, it required caution to find a section of tendon which did not have visible small blood vessels on or near the surface. It was a constant challenge to find a 2 mm area which appeared clear of vessels. It was much less difficult in the dog and goat since their Achilles tendons have a greater length free from muscle fibers.

The joint capsule selected for study was just above the insertion of the Achilles tendon. Dissection of the surrounding tissue was not technically difficult. There tended to be a modest amount of blood which seeped from the area during manipulation but it was possible to find an area free from obvious vessels. The joint capsule surgery was performed as follows: The capsule was carefully exposed to remove as much of the overlying fascia and connective tissue as possible so that there would be as little extraneous tissue as possible between the probe and capsule. The probe holder was then sutured to the surrounding tissue, avoiding any damage, pressure, or stress to the capsule itself.

Measurements were recorded as described above, including shifting the probe to the flank to provide a comparison of flow with skin which has undergone extensive blood flow studies for a number of years.

Upon completion of the study each animal was euthanized with an overdose of Nembutal, and in some instances exsanguinated following death.

## RESULTS

When the laser was first placed on the tendon, perfusion initially was at an elevated level. But after about 5 min, perfusion tended to drop to a lower level where it then took up a rhythmic change, but in general remained constant for extended periods. This trend suggests that manipulation of the tendon caused a transient increase in blood flow which temporarily masked the uninsulated, normal perfusion pattern.

A typical blood flow pattern in both the joint capsules and tendons of all of the animals showed what appeared to be a fairly rhythmic fluctuation of blood flow, but even after as long as 6 hours of continuous observation, and indeed until death, perfusion was never observed to drop to zero flow even if the animal was heavily anesthetized and there was no perceptible movement. Usually, flow tended to change on about a 14-18 min cycle if the animal was quiet and at a deep plane of anesthesia. The changes varied from a low of as much as 18% below the mean to a high of 25% above the mean when no heating or trauma was introduced (Fig. 1).

No two animals showed identical perfusion characteristics during the baseline period even when care was taken to minimize surgical trauma. Each subject had a different tendon flow pattern both in terms of the volume of flow (as measured by blood cell velocity), and each had its own cyclic rate of increased and decreased flow. The cyclic change was such that it would be elevated or reduced for 1-2 min, but return to a more stable flow for a period of from 10-15 min.

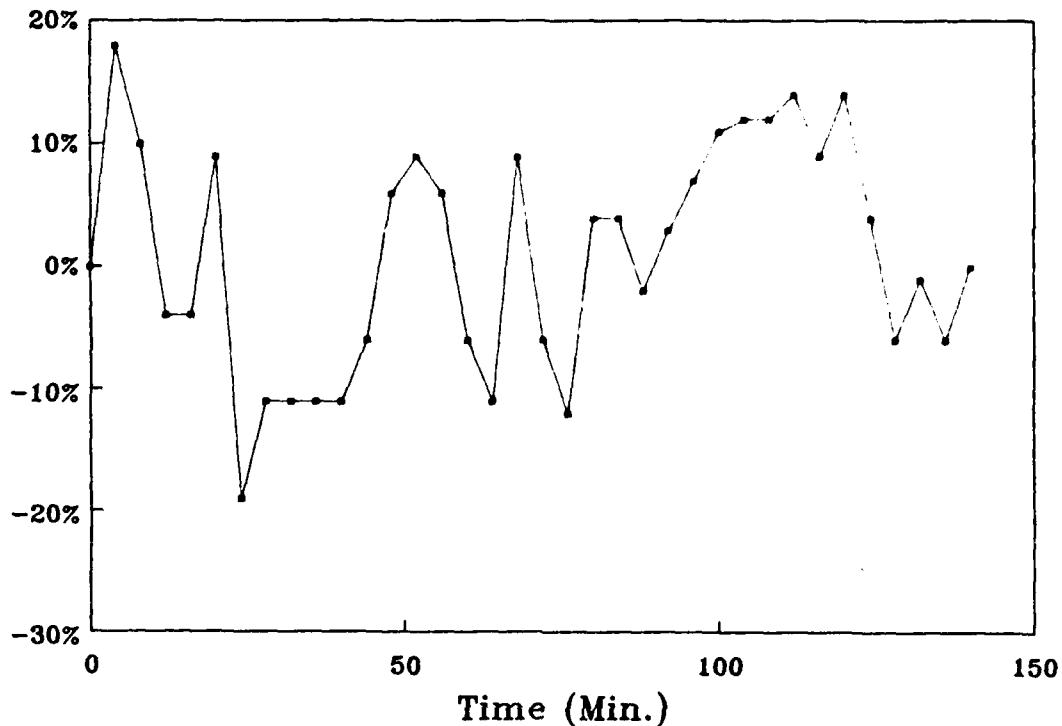


Figure 1. Pig tendon perfusion, control.

Damber et al. (23) found that, in the rat testis, perfusion was independent of both respiration and heart beat. In our work with pigs, goats, and dogs, it was observed that tendon perfusion was synchronous with respiration, but no pulsatile flow related to heart beat could be observed. This observation does not confirm that such pulsatile flow does not exist. Our device has a time constant of 5 s and updates the flow reading every 2 s.

This time constraint would mask the effects of pulsatile flow if it did exist. In some instances the effect of respiration was more noticeable than in others, and was not always related to the force of respiration, but at no time did the flow rate fall even close to zero.

In an effort to assure that perfusion was taking place, it was decided to introduce adrenalin into the circulation. In this way, if the perfusion decreased it would be proof that there had been perfusion up to that point.

The effect of Adrenalin on tendon blood flow followed the results obtained by Damber and his co-workers (23). An IV injection of 0.1 mg of adrenalin chloride resulted in a 25 to 37% decrease in tendon perfusion for from 15-20 min (Figs. 2,3), while an IV injection of 0.03 mg, either caused no change in perfusion, or in some instances resulted in a 5% increase for approximately 2 min followed by a slight decrease of approximately 10% for an additional 10 min before returning to the baseline flow rate.

A high dose of epinephrine causes a major vasoconstriction which reduced tendon capillary perfusion that cannot be overcome even by the rise in blood pressure and the great increase in heart rate.

An effort was made to repeat Hills' (3) observations which he carried out on guinea pig tendon blood flow. This experiment was accomplished by a study of the tendon of the commercial pig, using filtered carbon black, following his technique. It was found in our studies that some areas of the tendon showed the presence of carbon black within 10-15 s after injection in an ear vein, although not all of the tendon showed uniform uptake. However, the injection of filtered carbon particles resulted in significant pulmonary distress in the animals which was not overcome for a period of 10-15 min, and in one instance resulted in the death of the animal. During this time of distress, the laser indicated a sharp drop in perfusion of the tendon. Within 10 min, the distress usually disappeared and the tendon blood flow returned to pre-injection values. There is little doubt that this injection caused embolization of the pulmonary bed.

To overcome the pulmonary hypertension and embolization, another series of studies were carried out in which an aqueous solution of fluorescein dye was injected into the venous circulation via an ear vein. This dye has no apparent direct effect on the pulmonary circulation but can be visualized by ultraviolet light. Thus, it was possible by this method to directly observe the presence or absence of tissue perfusion.

We observed that the dye appeared in the tendon within 10-20 s in selected surface areas 4-5 x 1-3 mm. However, when the tendon fibers were spread it could be seen that the dye had, in some instances, extended for more than 3 mm along a number of fibers which were not visible from the surface. In fact, at the end of 10 min, if the tendons were removed and examined under ultraviolet light both in cross section and in length, they revealed that all fibers of the entire tendon were stained with fluorescein.

Further, in repeated instances fluorescein, which was injected into an ear vein, appeared first at the distal and proximal ends of the tendon, converging toward the middle. This tendency indicates that the perfusion of the tendon simultaneously comes from its origin and insertion ends.

It was considered unlikely, but possible, that in every instance the capillary beds happened to be open at the time fluorescein was infused. To see if the bed closed down later, we followed the fluorescein 10 min later with an infusion of an aqueous solution of brilliant blue. Under room light the brilliant blue dye appeared in the tendon within 10 s, and within 5 min had completely stained the tendon. With the room darkened the fluorescein still could be observed under ultraviolet light.

The rapidity with which the tendon appeared to sequester the carbon particles and the two dyes was puzzling in light of Hills' observations. For this reason, the fluorescein study was repeated with the simultaneous measurement of blood flow by the laser-Doppler. This observation presented a paradox. As already stated, within 10 s after injection of the fluorescein, some of the tendon fibers showed the appearance of the dye, probably reflecting the circulation time. Of some importance was the observation that, during the time the dye was gradually appearing on the surface of the tendons, the laser-Doppler showed that there was a constant level of perfusion with no

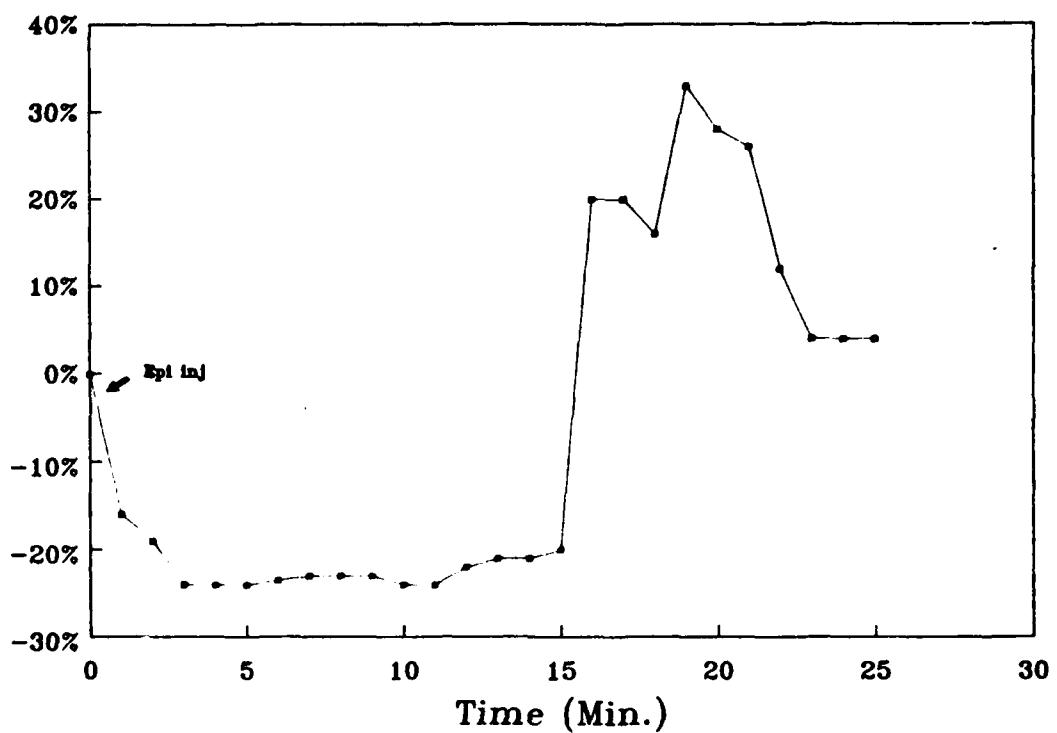


Figure 2. Changes in joint perfusion after Adrenalin infusion in the pig.

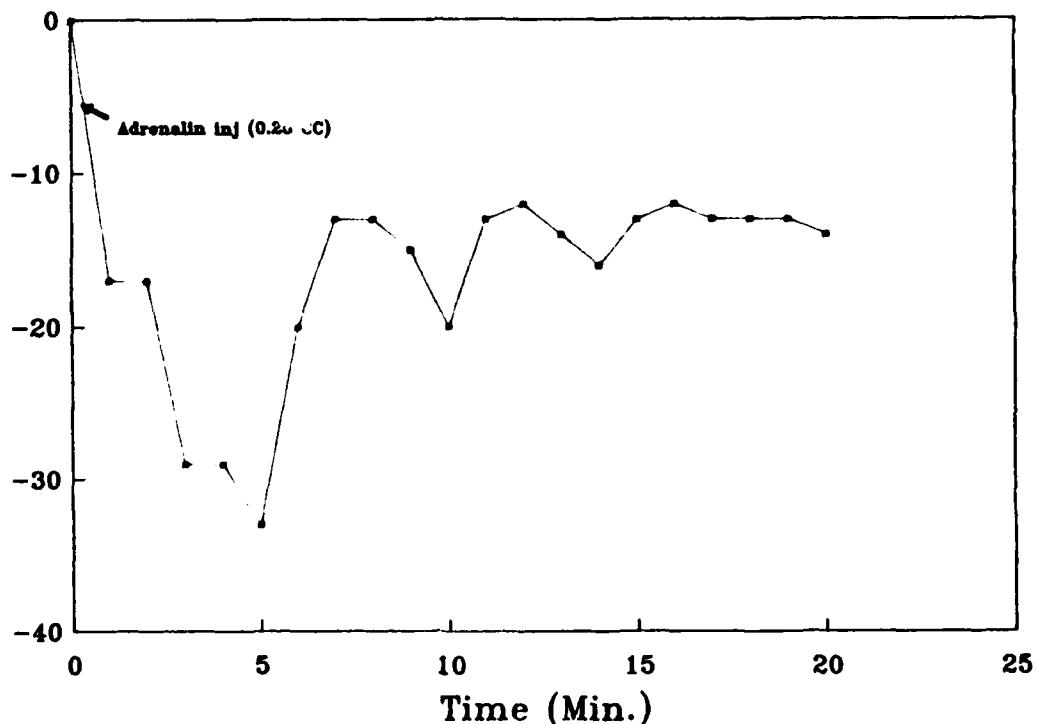


Figure 3. Changes in tendon perfusion in the pig after Adrenalin infusion.

evidence of perfusion shutdown and, indeed, in the absence of the carbon injection there was no significant change in flow rate.

Joint capsule perfusion was carried out in a manner identical to that of tendons. In all three species the fluorescein appeared within 1 min, and within 10 min the entire capsule showed a fairly uniform distribution of the dye when viewed under ultraviolet light.

The effects of heating varied greatly between animals. Temperature was raised by a local application either from a heated ring around the laser-Doppler probe, or by a heating blanket directly around the local area. The temperature was usually raised to 42-47°C. In most instances there was a 10-20% increase in flow when the limb was heated in the vicinity of the tendon, but in 3 instances the change in perfusion appeared to be insignificant (Figs. 4,5, and 6).

Table 1 presents the mean percentage elevation of perfusion in both joint capsule and tendon as a result of local heating in the three species of animals studied. Due to the great variation in the response to heat it can only be said that heat does, on the average, increase perfusion in both joint capsule and tendon in all of the animal species tested. However, there was such a range of response that statistically valid conclusions could not be drawn between species or between tendon and joint perfusion. This range is illustrated in Table 2, which shows the percentage of change in joint capsule perfusion in 9 pigs during local heating. It can be seen that it ranged from a low of 23%-180% above control values.

TABLE 1. AVERAGE PERCENTAGE INCREASE IN PERFUSION DUE TO HEATING

Species	X	n-1
Pig	60.7	+ 61.1
Dog	151.7	+155.7
Goat	87.3	+ 97.6

where: X = Mean  
n-1 = Standard Deviation

A study of Figures 4 and 5 reveals an unexpected phenomenon. In both instances the perfusion was elevated, but that the perfusion decreased while the heat still was being applied. This was a common finding. Further, as seen in Figure 6, after the heat had been removed and the tendon or joint had cooled to approximately body temperature, perfusion sometimes increased to an even higher level than during the heating phase.

It will also be noted in Figure 7 that at a temperature of 47°C., the perfusion was raised by 80% where it remained for 27 min with minor fluctuation of 1-2%. Then it precipitously dropped to 23% of the initial flow prior to heating. This precipitous drop was seen on a number of occasions. Since there was no change in the anesthetic level, probe orientation or animal movement, it can only be concluded that this change represented control by the autonomic nervous system.

TABLE 2. PERCENTAGE MAXIMUM INCREASE IN PERFUSION OVER CONTROLS  
IN JOINT CAPSULES OF 9 PIGS

Percentage Rise in PU	
56	
180	$n-1 = 49.9$
26	$Ex^2 = 57800$
40	$X = 65.6$
63	
63	
23	
100	
39	
590	

where:  $n-1$  = Standard deviation  
 $Ex^2$  = Sum of squares  
 $X$  = Mean

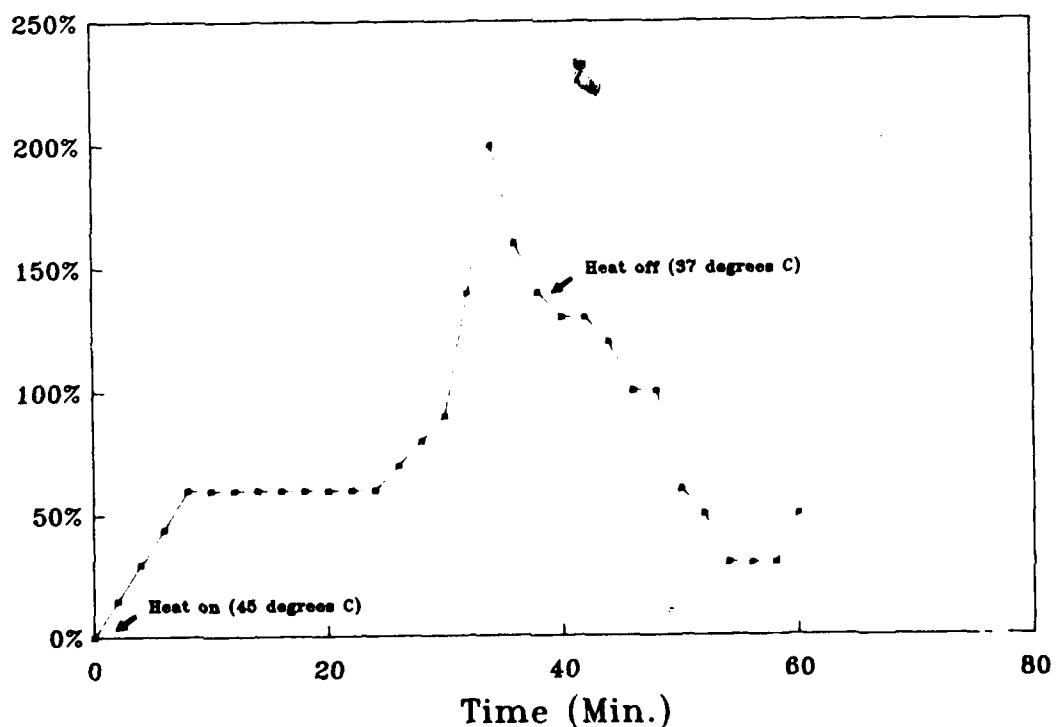


Figure 4. Changes in perfusion in the goat tendon after application of heat.

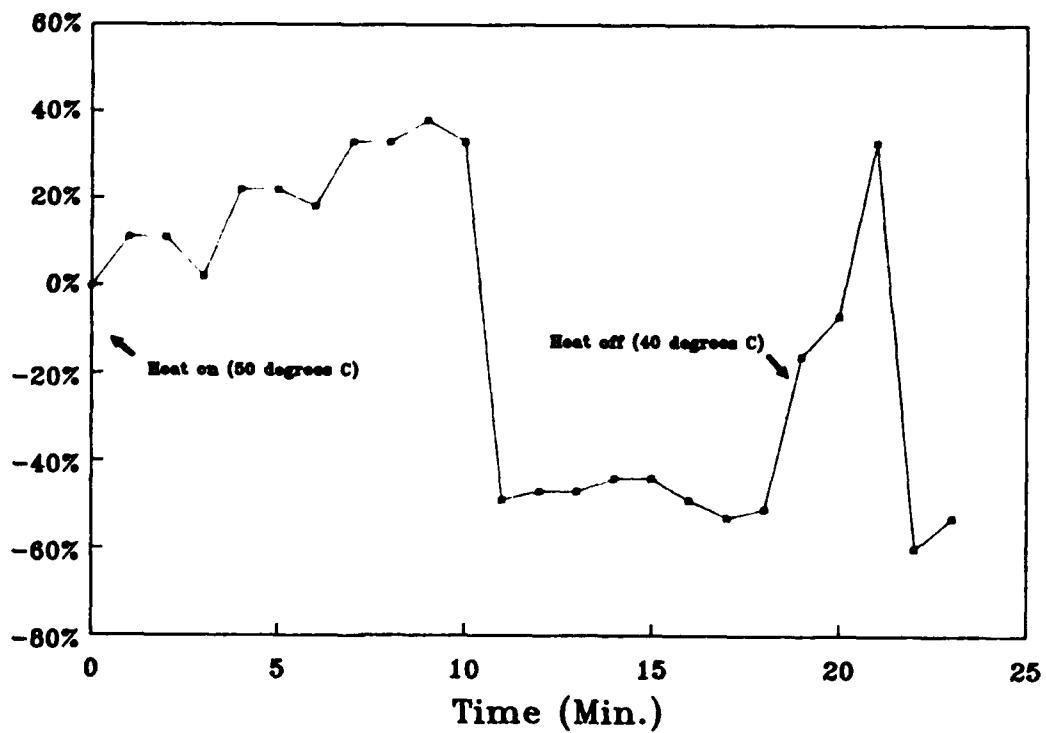


Figure 5. Changes in perfusion in the pig joint after application of heat.

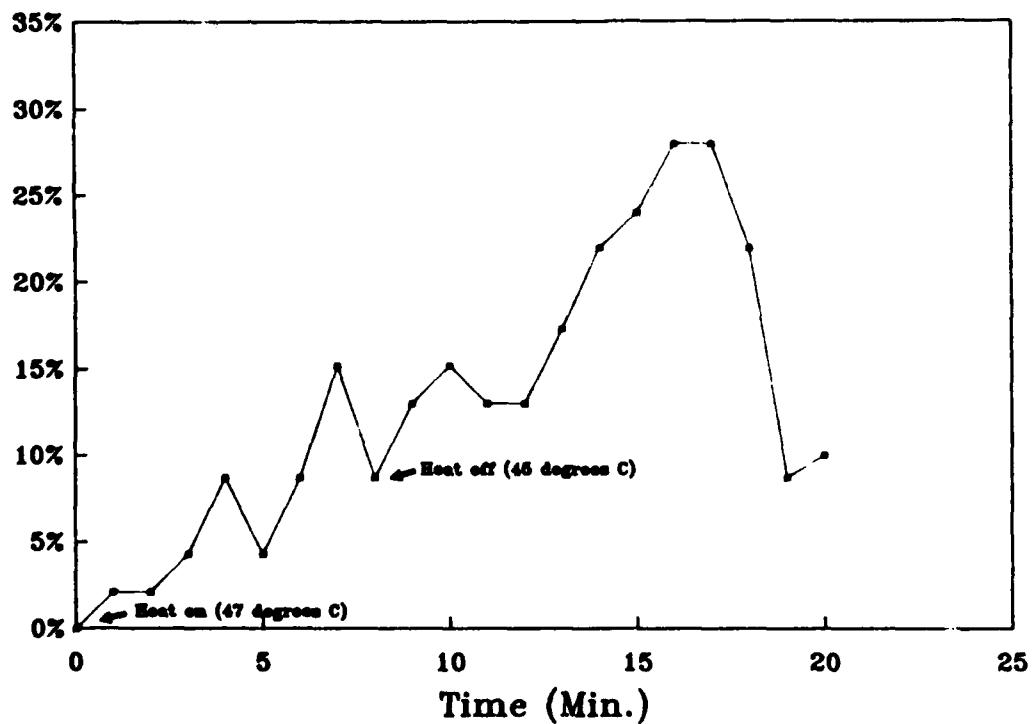


Figure 6. Changes in tendon perfusion in the goat after application of heat.

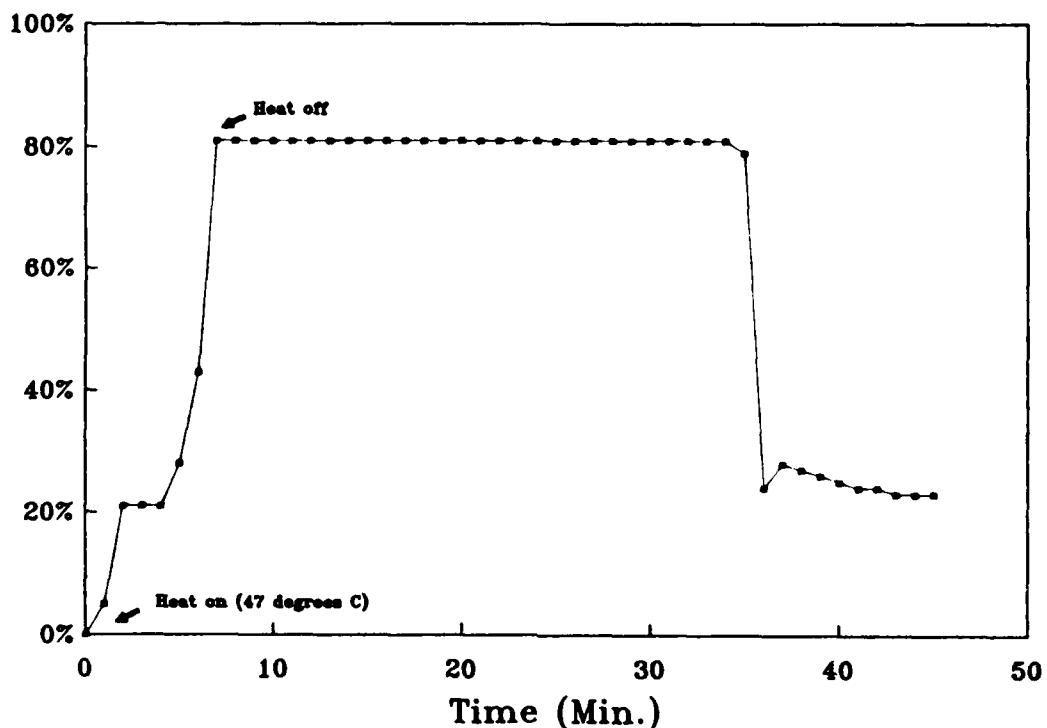


Figure 7. Changes in tendon perfusion in the dog after application of heat.

#### DISCUSSION

It must be recognized that the laser-Doppler blood flow measuring device does not directly provide a quantitative measure of the actual blood flow. Rather, like all other Doppler devices, it measures the velocity of the blood cells or other moving substances from which the light or ultrasound can reflect (24,25). However, since the approximate area of tissue being observed can be assumed, it may be possible to quantitatively approximate perfusion. This measurement method must be considered with caution because in some tissues there is a reversal of flow in capillary beds (24). It should be noted, however, that Field (2) observed that while there were some anastomoses, most of the blood flow was in a longitudinal direction with reference to the orientation of the tendon fibers. This perception was also born out by our observation concerning the proximal and distal uptake of fluorescein followed by a longitudinal movement of the dye along the tendon. We were unable, however, to quantify perfusion in either the joint or tendon by the laser-Doppler system.

One great advantage of laser-Doppler flowmetry is that perfusion of a small, finite area can be measured continuously without the need for the probe to contact the tissue, and without significantly insulting the circulation other than the initial manipulation to isolate the joint or tendon from its surrounding tissue. This method avoids the limitations of microspheres or

other particulate substances which interrupt the flow, and thus provides only a one-time, instantaneous reading. It also avoids the introduction of radioactive ions ( $Rb^{86}$ ,  $I^{131}$  (Sapirstein (26), Fife (27)) which are terminal studies, and again provide only one-time values. The radioactive gas, such as  $Xe$ , does permit repeated measurements, but because of the relatively large size of the field of the probe, it would be difficult to limit observation to an area as small as 1 mm in diameter required in the study of tendon perfusion. The xenon method covers a large area, and thus would also tend to see blood flow in the surrounding tissues. Thus, the laser-Doppler method avoids the use of radioactive isotopes as well as methods which interrupt or re-direct either the arterial or venous blood supply (5,9).

All results are reported in Perfusion Units (PU) or in percentage changes in perfusion units. Perfusion Units are a factor of the blood cell velocity times the number of cells in the field. Therefore, while this does not make it possible to provide a quantitative value, the relative changes can be accurately reported as percentage of change from any initial established baseline value. Perhaps more important for the present study, we can positively measure the absence of flow since a zero PU reading can only be obtained when there is no movement of blood cells within the field being monitored. We also can positively see the effects of heating on perfusion both in joint capsules and in tendons.

Before results could be evaluated it was first necessary to determine the reliability, stability, and limitations of this laser-Doppler system and thus, the validity of the measurements. To accomplish this validation, blood flow was measured in Silastic 1-mm tubing, using a Harvard pump to create blood flow. Blood was taken from a sibling which had been used in a previous perfusion study to assure similar hematocrit and other blood components. Blood flow was directly measured for each pump setting to calibrate the pump-syringe system.

It was found that at a high flow rate (well above the accepted physiological levels), the measurements became less reliable due to the saturation of the cut-off system in the laser-Doppler unit. However, at the blood flow levels found in this study, the unit which was employed was shown by Overaasen to be linear (14).

When the blood flow was interrupted by removing the tendon section the perfusion units dropped to zero, but if left intact in the dead animal, the PU units often dropped to 1 or 1.5 for an extended period. This drop in PU was not unexpected since it was probable that even when there was no cardiac output, the capillaries would have some perfusion for a number of minutes as the blood moved from the arterial side to the venous side to equalize vascular pressures. This was not a serious problem in our quest for the answer to joint or tendon perfusion because the very low PU values at this time were usually no more than 0.1% of the flow values seen throughout the study. Thus, if this value were subtracted from the values observed during the study, it would not significantly alter the fact that continuous blood flow appeared to be present both in the tendon and joint capsule. Initially, we considered the possibility that this continued low PU value was due to Brownian movement since the developers of the system raised this possibility. However, it is more likely that it was due to the blood pressure equalization

between the arterial and venous vessels since the perfusion units dropped to zero or 0.2 PU units when the tendon was removed, but still contained stagnant blood.

The significance of collateral or reversed blood flow in the capillary beds of tendons and joint capsules must be considered. It should be noted that when fluorescein was injected, the uptake in the tendon began at each end of the tendon and converged somewhat linearly toward the center suggesting that the major direction of blood flow is along the tendon fibers. Since the dominant flow is also from arterioles to venules, any perfusion would tend to contribute to gas exchange, involving both perfusion and diffusion of inert gas. Further, it is clear that the laser-Doppler unit measures perfusion in a volume of about  $3 \text{ mm}^3$ . It would appear, therefore, that even though some capillaries in this area were closed down, if perfusion continued in other capillaries within this same volume the diffusion of inert gas would make it possible for such gas to be removed with little problem during decompression.

It is important to determine if blood flow is interrupted for long periods of time in large areas, or if the areas of interrupted blood flow are very small, permitting diffusion to take care of the supersaturation of inert gas. In fact, our evidence suggests that perfusion is not completely interrupted at all. The drop in capillary perfusion with the infusion of epinephrine further confirms this observation (Figs. 2,3). Secondly, the question must be raised whether the natural production of epinephrine or norepinephrine related to the autonomic nervous system or the effect of stress on the adrenal medulla, may cause a similar reduction in tendon and joint blood flow. If this is the case, it may be important to consider if such increases in catecholamines could reduce tendon or joint perfusion, and thus interfere with inert gas elimination. It might also be considered whether exercise, stress or other causes of increased catecholamine release may result in a decreased perfusion of the joint capsules and tendons and thus, alter inert gas elimination. On the other hand, it appears that direct manipulation of the tendon resulted in a temporary increase in perfusion.

It should be noted that when the laser probe was repositioned, there was always a small difference in the perfusion units observed. This variance should not come as a surprise since each time the probe is moved it focused on a different geometry of capillaries. Thus, it would be expected that there would be a different reflection based on the number of red blood cells which were in the observed field. If anything, this difference would support the accuracy of this method. While some positions of the probe would invariably see shunts, arterioles and venules, it is not likely that this would be the case in every position. In 60 individual examinations of tendons, there was no single instance in which the probe showed an absence of flow.

It is possible that some of the changes in perfusion which were synchronous with respiration actually reflected a change in perfusion due to change in blood pressure related to the respiratory cycle. As shown in man by Nilsson et al. (15), a deep inspiration caused a transient vasoconstriction in peripheral capillary beds in the skin which would return to normal after about 10 s, even though the subject continued to hold his breath. The fact that a similar pattern appeared in the tendon blood flow in our animals suggests that tendon and joint capsule perfusion is also influenced on a short-term basis by the autonomic nervous system. Further evidence that the autonomic

nervous system affects capillary perfusion may be seen by immersing one hand in cold water, resulting in blood flow in the contralateral hand being reduced by about 75% (15).

The possible effect of respiration and the autonomic nervous system on the laser-Doppler method of measuring blood flow was further considered. This effect was studied in two ways using the pig model. In the first method the animal was heavily anesthetized to the level at which the respiratory center was paralyzed and caused respiration to cease. In the second method, 0.4 units per pound of tubocurarine chloride were slowly injected via an ear vein to paralyze the neuromuscular junctions of all voluntary muscles. When respiration ceased, the animal was immediately placed on a ventilator. It was noted that with the respiratory muscles functioning normally, inspiration would cause a drop in intrathoracic pressure which would tend to aid venous return to the chest and heart, and at the same time momentarily reduce the blood flow out of the chest. The cyclic changes in tendon blood flow were synchronous with the artificial ventilation.

When positive pressure breathing was instituted, the inspiratory phase raised the intrathoracic pressure and thus aided in ejection of blood from the heart and chest. This should cause perfusion to rise at the same time. During the exhalation phase, when the intrathoracic pressure was lowered, blood could more easily enter the heart, but the cardiac output would momentarily drop as would perfusion.

It is probable that the pressure changes due to the use of the ventilator should have no direct effect on the autonomic nervous system. Further, when the respiratory center is anesthetized, the autonomic nervous system should not play a significant role on perfusion. Although this rationale may be subject to varying interpretations, if our hypothesis is true, it appears that much of the rhythmic changes seen in tendon and joint blood flow can be explained by changes in cardiac output.

Since the laser-Doppler actually detects movement of surfaces, the question still must be raised as to the possibility that some of the blood flow readings were actually due to gross movement of the area being observed, and not to movement of blood cells. This movement was studied in two ways as follows:

First, the gastrocnemius muscle in a dead animal was slightly moved by direct manipulation. It was possible to create PU values nearly identical to those produced in the live animal by such movement, even in the absence of perfusion, although it required much greater movement than was the case during any experiment. However, it was apparent by this that a sufficiently gross movement of the tendon could be confused with perfusion values or added to them, and thus, give a falsely high perfusion value when little or no perfusion actually existed.

To differentiate between the tendon PU values created by movement and those created by actual perfusion, the animal was allowed to die. When the heart stopped, the animal was placed on the respirator. When respiratory movement was initiated by the respirator, the tendon showed a rise of only 1-2 PU, above the resting baseline seen during apnea. When the probe was placed on the flank, perfusion rose to between 8-12 PU and the excursion was much greater. When the positive pressure ventilation was stopped the perfusion

units fell to between 0 and 1 PU in both locations. This variation was not surprising because when positive pressure ventilation was applied to the animal, the flank moved in consonance with the respiratory cycle, but did not achieve as high PU values as when the animal was alive.

This observation was repeated by the use of curare which paralyzed all neuromuscular junctions, and thus stopped all muscle movement. While the animal was on the respirator there were small excursions of 1 or 2 PU units which were synchronous with the respiratory cycle. However, when the respirator was halted the perfusion dropped by between 2-10 PU, but did not approach zero. If apnea was allowed to continue, the perfusion began to drop significantly, but this reduction was the result of cardiovascular insult due to hypoxia.

It appears, therefore, that the laser-Doppler unit will not distinguish between tissue movement and actual perfusion. This would be a greater problem when measuring blood flow near the chest or abdomen, or in any tissue which is subject to physical movement. However, this movement apparently has little effect on the measurement of tendon or joint capsule blood flow if the animal is breathing quietly without limb movement either due to a deep plane of anesthesia, or due to the use of curare which paralyzes the neuromuscular junctions and causes voluntary muscles to become flaccid.

Our studies with fluorescein dye do not support the observation reported by Hills (3). At no time during 27 studies were we able to observe zero perfusion in either pig, dog, or goat tendon or joint capsules. We did, however, note a cyclic flow pattern but the cycle times were significantly different from those reported by Hills. While he reported that some areas of tendons did not take up the tracer for many minutes or hours, the fluorescein in our studies was present within 10 s in some areas, and in every instance was taken up by all parts of the tendon within between 5 and 10 min. The first appearance of the dye began at the insertion end of the Achilles tendon, spreading rostrally. Within 1-2 min the dye was also observed spreading from the rostral end of the tendon, finally meeting near the center of the tendon.

It was clear in our studies that although the uptake of the dye was not instantaneous in all parts of the tendon, the laser-Doppler showed that there was continuous perfusion in the area being observed. This observation raises the question why Hills observed a mottled effect in his tendons. One possible explanation may be that the surface of the tendons in his studies may have had severe vasoconstriction due to drying or to the reduced temperature caused by exposure to the air. If this were the case, then it might be possible that the dye would not appear as readily in surface capillaries and would be invisible to the eye. In our studies, every effort was made to keep the tendons and joint capsules covered with skin except directly under the probe. On the other hand, the fact that the tendons in his studies eventually sequestered the dye would suggest that most of the surface capillaries eventually did open up, or perhaps became patent again after microembolization of carbon was resolved.

The possibility was also considered that the difference between our work and Hills' may have been that the tendon was locally heated by the laser beam, and thus had a level of vasodilation which resulted in that small area being continuously perfused selectively. To clarify this possibility, we removed the laser beam just as fluorescein was injected. The dye did not

appear at the site of the Doppler probe any sooner than in the surrounding tendon which had not been under observation. This would suggest that the area directly under the probe was not receiving an increase in perfusion due to the probe. None of the other workers who have used the laser-Doppler device have reported any vasoactive effect due to the laser itself.

The difference between our observations and those of Hills naturally raises the question as to the validity of the laser-Doppler device as well as on Hills' methodology. Since the longest time constant used in this device was 3 s, it is conceivable that a shorter event might go unnoticed. However, we have found no references to shifts in perfusion which are rapid enough to be completely masked by a 3-second time constant. Hills measured his changes in many minutes and even in hours. Further, it should be noted that when the probe was removed from the tissue, the PU quickly fell to zero, thus indicating that it had, indeed, certainly been observing blood cell movement.

Further confirmation that there was truly a continuous perfusion in the tendons was demonstrated by the use of epinephrine. When epinephrine was injected, there was invariably a significant decrease in perfusion units. If the blood flow had already been zero, there could not have been a further significant decrease as was observed.

Finally, when the heart stopped and perfusion ceased, the PU always decreased to near zero, showing that there had been perfusion before.

The question of the possible overlap in perfused areas needs to be considered. It might be suggested that the area covered by the laser-Doppler probe was so great that it overlapped perfused and non-perfused areas, and thus might show a constant perfusion, whereas some portion of the area being observed actually did have zero flow as Hills described. We do not believe that this is the case since the laser field of view is only about 1 mm wide. This is approximately the area observed by Hills, although he was only able to see the surface, whereas the laser is able to penetrate the tissues to a depth of approximately 1 1/2 mm. It would not be likely that the laser would often average both perfused and non-perfused areas within this small area of observation.

Still another possible problem with Hills' observations may be that he used carbon particles as markers. We found that the injection of even filtered carbon particles resulted in a significant amount of pulmonary hypertension which in some instances was transient, but it is likely that this would cause some alteration of the autonomic nervous system. Fluorescein does not cause pulmonary hypertension and should have no effect on the autonomic nervous system. The interesting paradox between the perfusion as measured by laser and by the carbon particles is still not completely resolved.

It is possible to measure blood flow semiquantitatively by the ultrasonic Doppler method if the probe is placed around a vessel which has a known internal diameter. We have considered the possibility of quantifying perfusion by the laser-Doppler method by making an assumption concerning the volume of tissue which the laser beam sees. At this time, however, we are not ready to present this possibility because of the complicating factors inherent in such a method.

## CONCLUSIONS

It is clear that the use of hydrogen washout to measure perfusion in tendons and joint capsules is not a satisfactory method given the present state of the arts, although with further technical development it probably could still be made to work in tissues with such low perfusion. At this time we would place no reliance on such perfusion measurements for these areas, although this method appears to be satisfactory for other tissues which have higher perfusion (11).

The use of the laser-Doppler method for measuring perfusion requires caution and careful control, primarily to avoid tissue movement. Further, at this time such flow cannot be quantified. However, percentages of change in flow under various altered physiological conditions can be reasonably carried out and their reliability appears to be within the limits of biological variability. The greatest weakness in this method appears to be the possibility of confusing tissue movement with actual red blood cell movement. In the present work this was minimized and essentially eliminated by the use of deep anesthesia or by curare which produced flaccid paralysis eliminating all voluntary muscle movement.

We could not confirm the observations by Hills, in which he reported that tendon blood flow was shut down for extended periods of time in many areas of the tendon, sometimes for up to 4 h. In our study, it appeared that there was constant but low perfusion of the tendons and joint capsules in the three species of animals tested, and that perfusion appeared to be rather uniform throughout each tendon or capsule. As expected, we could see no statistically significant species difference between the three used for this study.

Although local heating did elevate the perfusion by as much as 300%, this increased perfusion was not consistent in all instances. It would appear that exercise and manipulation caused a larger increase in perfusion than did heat alone.

The question was initially raised as to the possibility that tendon and joint perfusion may be responsible for the extremely long oxygen pre-breathing time required to protect the astronaut or pilot against altitude decompression sickness. Since the laser beam observed a volume of approximately 1 mm<sup>3</sup>, and at no time did we see zero flow, it appears that even if there was a local shutdown of perfusion in a small area adjacent to the one being observed, the perfusion in adjacent areas (within 1 mm) is sufficient to prevent supersaturation with inert gas, and hence the formation of bubbles in these tissues. We hasten to note again that we have no evidence that there are such areas in which perfusion does not exist. We were unable to find any.

Based on our present study, although we can not positively eliminate the possibility that tendons and joint capsules are the cause of bends found in astronauts and pilots, from our data it is highly unlikely. Further, while there is no doubt that a 4-h prebreathing of oxygen is required to assure freedom from bends, we believe that the cause of these bends must be found elsewhere.

#### REFERENCES

1. Waligora, J., Horrigan, D., Conkin, J., and Gilbert, J. Incidence of symptoms and venous gas bubbles in male and female subjects after decompression. Aviation, Space, and Environmental Medicine Scientific Meetings May 1986, p 511.
2. Field, P. Tendon fibre arrangement and blood supply. *Aust N Z J Surg* 40:298-302 (1971).
3. Hills, B. Intermittent flow in tendon capillary bundles. *J Appl Physiol: Respirat Environ Exercise Physiol* 46:696-702 (1979).
4. Krogh, A. The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion. *J Physiol (London)* 52:391 (1918).
5. Burton, K., and Johnson, P. Reactive hyperemia in individual capillaries of skeletal muscle. *Am J Physiol* 223:517-524 (1972).
6. Johnson, P., and Wayland, H. Regulation of blood flow in single capillaries *Am J Physiol* 212:1405-1415 (1967).
7. Guyton, A. *Textbook of Medical Physiology*, Fourth Edition, 1981. W.B. Saunders Co. Philadelphia, London.
8. Eriksson, E., and Lisander, B. Low flow states in the microvessels of skeletal muscle in cat. *Acta Physiol Scand* 86: 211-222 (1972).
9. Fick, A. Ein neuer blutwellenzeichner. *Arch f Physiol* 583 (1864).
10. Aukland, K., Bower, B., and Berliner, R. Measurement of local blood flow with hydrogen gas. *Circ Res* 14:164-187 (1963).
11. Yimenez, G. The influence of hyperbaric oxygen therapy on perfusion of tissues. Dissertation: Texas A&M University, 1978.
12. Nilsson, G., Tenland, T., and Oberg, P. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans on Biomed Engineering* BME-27:12-19 (1980).
13. Kvernebo, K., Lunde, O., Strand, E., and Larsen, S. Human gastric blood circulation evaluated by endoscopic laser doppler flowmetry. *Scand J Gastroenterol* 21:685-692 (1986).
14. Overaasen, M. (Thesis) University of Trondheim, Norway. 1985.
15. Nilsson, G., Tenland, T., and Oberg, P. Evaluation of laser doppler flowmeter for measurement of tissue blood flow. *IEEE Transactions BME-27:597-604* (1980).
16. Sundberg, S. Acute effects and long-term variations in skin blood flow measured with laser doppler flowmetry. *Scand J Clin Lab Invest* 44:341 (1948).

17. Swiontkowski, M., Tepic, S., Gantz, R., and Perren, S. Laser doppler flowmetry for measurement of femoral head blood flow. *Helv Chir Acta* 53:55-59 (1986).
18. Shepherd, A., and Riedel, G. Differences in reactive hyperemia between the intestinal mucosa and muscularis. *Am J Physiol* 247 (Gastrointest Liver Physiol 10) (1984).
19. Hassan, A., Rayman, G., and Tooke, J. Effect of indirect heating on the postural control of skin blood flow in the human foot. *Clinical Science* 70:577-582 (1986).
20. Eriksson, E., and Myrhage, R. Microvascular dimensions and blood flow in skeletal muscle. *Acta Physiol Scand* 86: 211-222 (1972).
21. Nilsson, G., Tenland, T., and Oberg, P. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans Biomed Eng* 27:1-12 (1980).
22. Wilander, L., Rundquist, I., Oberg, P., and Odman, S. Laser He/Ne light exposure of red blood cells. *Med Biol Eng Comput.* 558-560. Sept, 1986.
23. Damber, J., Lindahl, O., Selstam, G., and Tenland, T. Testicular blood flow measured with a laser doppler flowmeter: acute effects of catecholamines. *Acta Physiol Scand* 115:209-215 (1982).
24. Stern, M. In vivo evaluation of microcirculation by coherent light scattering. *Nature (London)* 254:56-58 (1975).
25. Riva, C., Ross, B., and Benedek, G. Laser doppler measurements of blood flow in capillary tubes and retinal arteries. *Invest Ophthalmol II:* 936 (1972).
26. Sapirstein, L. Cerebral blood flow in rats. *Am J Physiol* 193:1 (1958).
27. Fife, W. The fate of radioactive iodine after parenteral administration. *Dissertation, Ohio State University, 1962.*